Purkinje cells in the lateral cerebellum of the cat encode visual events and target motion during visually guided reaching

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In this study the receipt of visual information by the lateral cerebellum and its contribution to a motor output was studied using single unit recording of cerebellar cortical neurones in cats trained to perform visually guided reaching. The activity of Purkinje cells and other cortical neurones in the lateral cerebellum was investigated in relation to various aspects of the task, such as visual events, parameters of target movement, and limb and eye movements. Two-thirds (66%) of Purkinje cells tested could signal simple visual events, such as a flash of light. Neurones were also capable of detecting other less potent, but behaviourally important visual events, such as a 'GO' signal (LED brightening). Half of the cells tested were responsive to the on-going motion of the visual target, displaying tonically altered discharge rates for as long as it was moving, and a 'preferred' target velocity. A small proportion of cells showed short latency visual modulation that persisted during the forelimb reach. Anatomical tracing studies confirmed that the recordings were obtained from the D1 zone of crus I. In summary, cells in this region of lateral cerebellar cortex perform simple visual functions, such as event detection, but also more complex visual functions, such as encoding parameters of target motion, and their visual responsiveness is appropriate for a role in accurate visually guided reaching to a moving target.

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Vision is extremely important for the accurate execution of many voluntary movements, and communication between visual and motor areas of the brain is therefore essential. The evidence that the cerebellum is the vital part of this link and plays an important role in the control of visually guided movement is anatomical, clinical and electrophysiological (Stein, 1986; Stein & Glickstein, 1992; Ebner & Fu, 1997). Massive inputs from cortical extrastriate visual areas and subcortical visual structures, routed via the pontine nuclei, arrive as mossy fibres terminating in the cerebellar hemispheres (reviewed by Stein & Glickstein, 1992). Anatomical and electrophysiological studies have also demonstrated that visual information is relayed via climbing fibre afferents to the lateral cerebellum (Hoffman *et al.* 1976; Akaike, 1986*a*,*b*,*c*; Edge *et al.* 2003).

The importance of these inputs for visually guided movement is betrayed by the profound deficit in visuomotor performance that results from their removal (Classen *et al.* 1995) or from cerebellar injury; accuracy of tracking is decreased, as is the ability to integrate ocular and other motor systems, and deficits in the speed of movement become apparent (Holmes, 1939; Miall *et al.* 1987; Vercher & Gauthier, 1988; Liu *et al.* 1999). Both anatomy and pathology therefore suggest a critical role for the lateral cerebellum in the visual guidance of movement.

Cells in the pontine nuclei that receive visual input are particularly responsive to visual events and to the velocity and direction of a moving target (Zeki, 1974; Baker *et al.* 1976; Thier *et al.* 1988). Thus, the activity of cells that forward information to the lateral cerebellum suggests that we should find encoded in the activity of their target cerebellar neurones the signalling of visual events and parameters of a target's motion; and there is some evidence that this is the case (Chapman *et al.* 1986; Marple-Horvat & Stein, 1990; Mushiake & Strick, 1993; Marple-Horvat *et al.* 1998). Much less is known of the role of climbing fibres in relaying visual signals to the lateral cerebellum; though it is clear that events such as a flash of light or appearance of a target (Marple-Horvat & Stein, 1990; Kitazawa *et al.* 1998) can be forwarded to the cerebellum by this route.

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A key feature of the cerebellum is its division into longitudinal olivo-cortico-nuclear zones, with each zone of Purkinje cells defined by their receipt of climbing fibres with similar receptive field characteristics originating from a discrete territory within the inferior olive and innervating specific groups of cells of the target efferent nucleus (Brodal & Kawamura, 1980; Voogd & Bigaré, 1980; Voogd & Glickstein, 1998; Apps & Garwicz, 2005). Each zone influences a different descending motor path, and many regard this as a fundamental feature of the cerebellar contribution to motor control (Ito, 1984; Apps & Garwicz, 2005). There are four zones in the lateral hemisphere: C2, C3, D1 and D2. The source of climbing fibres to the D1 zone within crus I arises from visual pathways (Edge *et al.* 2003). Thus, the objective of this study was to understand how visual inputs to the D1 zone of the lateral cerebellum might be used to guide movement. We show that the simple spike and complex spike activity of D1 zone Purkinje cells precisely signals visual events, and that simple spike activity also encodes target motion during visually guided reaching.

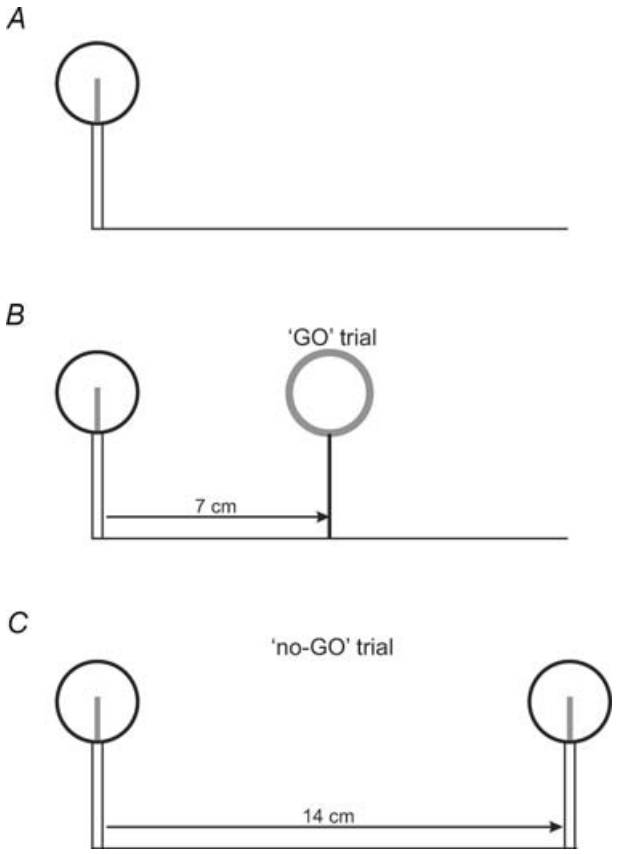


Figure 1. Schematic diagram of the sequence of events during the visually guided reaching task
A, a Perspex tube dimly lit by a ring of LEDs and containing a food reward is initially stationary 7 cm to the left of centre. *B*, in a 'GO' trial, the tube started to move horizontally in the rightwards direction at a constant velocity (3.1, 6.2 or 12.4 cm s^{-1}) across the cat's visual field. After an interval of 1.2, 0.6 or 0.3 s for slow, medium and fast velocities, respectively, the LEDs brightened to cue the animal to make a reach with its left forelimb, ipsilateral to the cerebellar recording site, to retrieve its food reward from the tube. The vertical rod was withdrawn by a solenoid to permit access to the inside of the tube. Within a block of 20 trials, the 'GO' signal was given in 10 randomly sequenced trials. *C*, in 'no-GO' trials, the 'GO' signal was not delivered, and the target continued to move in the rightwards direction, stopping 7 cm to the right of centre.

Methods

Experiments were performed on three purpose-bred adult male cats (4.5–5 kg) in accordance with local animal welfare guidelines and the UK Animal Scientific Procedures Act (1986). Cats were selected on the basis that they were amenable to handling and training and preferred to use their left forepaw for reaching. After selection, daily training sessions lasting up to 45 min were held for a period of approximately 8 weeks. In these sessions, the cats were trained non-aversively to sit quietly during presentation of a visual stimulus and to perform a visually guided reach–retrieval task with the left forepaw. After training, a recording chamber was implanted above the ipsilateral cerebellum under full surgical anaesthesia.

Visually guided reaching task

Each cat was trained to perform a visually guided reach towards a target (a Perspex tube of diameter 30 mm containing a food reward) travelling at a constant velocity rightwards in front of the animal. The tube was initially stationary 7 cm to the left of centre at a comfortable height for reaching and with its mouth dimly lit by a ring of LEDs (Fig. 1*A*). The entrance to the tube was blocked by a vertical stainless-steel rod a few millimetres behind its entrance. The tube then started to move at a slow (3.1 cm s^{-1}), medium (6.2 cm s^{-1}) or fast (12.4 cm s^{-1}) constant velocity rightwards across the cat's visual field (12.5, 25 or 50 deg s^{-1}, respectively). The cue to reach with the left forepaw ('GO' signal), consisting of a brightening of the LEDs and the withdrawal (by a solenoid) of the vertical rod, was given after an interval (1.2, 0.6 and 0.3 s for slow, medium and fast velocities, respectively) calculated so that addition of typical reaction and reach times meant that the target would be intercepted centrally in front of the cat (Fig. 1*B*). Entry of the cat's paw into the tube stopped its movement to permit retrieval of the reward. After a delay, the tube automatically returned leftwards to its initial position. Within a block of 20 trials, a 'GO' signal was given only in 10 randomly sequenced trials so that the cat did not know whether a reach for the reward would be permitted. In 'no-GO' trials, the 'GO' signal was not delivered; instead the target continued rightwards and stopped 7 cm right of centre (Fig. 1*C*), before returning to its initial location.

Surgery and implants

After an 8 week training period, the animals were prepared for chronic single unit recording in an aseptic operation under Propofol anaesthesia (0.05 ml min^{-1} I.V.; Schering-Plough, Welwyn Garden City, UK) following premedication with medetomidine hydrochloride (Domitor, 150 $\mu g\,kg^{-1}$ S.C.; SmithKline Beecham, Surrey, UK). A single dose of atropine (0.5 ml S.C.; Animalcare, Dunnington, UK) was given in order to prevent excessive secretion in the respiratory passages, and a wide-spectrum antibiotic (Amfipen LA, 0.2 mg kg^{-1} S.C.; Intervet, UK) was administered pre- and post-operatively. Throughout surgery, the temperature of the animal was kept within physiological limits with the aid of a thermostatically controlled heating blanket. Postoperative analgesia was administered (buprenorphine, 0.1 mg kg^{-1} I.M.; Reckitt and Colman, Hull, UK) and maintained for 24 h. In all cases, recovery was rapid and uneventful.

A small craniotomy was made to expose lobulus simplex and crus I of the left cerebellar hemisphere, and a small titanium recording chamber was placed over the four folia of crus I. Electro-oculography (EOG) electrodes (Clark Electromedical Instruments; Pangbourne, UK) were positioned on the bony orbital surfaces of the frontal sinuses, which were accessed by drilling keyhole slots in the skull surface vertically above. A stainless-steel T-bolt provided additional anchorage for the titanium chamber and served as an indifferent connection. A unipolar lead was implanted into the distal left forelimb to detect paw lift during reaching. Another lead routed down the back and bared for a 10 cm terminal section served as an earth. Bipolar EMG leads were also implanted into the ipsilateral triceps brachialis muscle to monitor EMG signals during rest and reaching. All leads (0.3 mm diameter Teflon-insulated multistranded stainless-steel; Cooner, Chatsworth, CA, USA) were fed subcutaneously to miniature connectors that were attached to the skull with dental acrylic cement, which was fashioned so as to incorporate the recording chamber, forming a lightweight headpiece. For additional details of the methods see for example Armstrong & Edgley (1984) and Marple-Horvat *et al.* (1998).

Recording arrangements

After recovery, recording sessions were held daily, and the activity of single cerebellar neurones was recorded extracellulary both whilst the cat performed the reaching task and whilst it sat quietly during presentation of visual stimuli. Parylene-coated tungsten microelectrodes (impedance 4–6 MΩ; World Precision Instruments, Stevenage, UK) were advanced using a microprocessor-controlled stepping microdrive mounted onto an *x–y* stage that was attached to the recording chamber. Extracellular single unit potentials were led from the microelectrode through a field effect transistor (FET) preamplifier to a unit amplifier (gain 1000×), filtered (bandpass filter 0.5–10 kHz) and led to an oscilloscope and audio amplifier. For each isolated unit, its size, duration, shape and co-ordinate were noted. Purkinje cells were identified by the presence of complex spikes and on the basis of their interspike interval distribution and spike duration (Eccles *et al.* 1966; Armstrong & Rawson, 1979). Units classified as mossy fibres displayed a triphasic appearance and wide dynamic firing range, varying from silence to high frequency bursts at several hundred impulses per second (Garwicz & Andersson, 1992). Putative Golgi cells were identified by their distinctive regular and low frequency discharge, and spike durations (Edgley & Lidierth, 1987; Vos *et al.* 1999). Cells which could not be categorized as any of the above were not classified.

Electromyogram signals were amplified (1000×) and filtered (bandpass 0.03–5 kHz). Electro-oculogram signals were passed through an isolating preamplifier (100×) and a high cut filter (500 Hz). Either no low cut or low cut at 0.1 Hz was applied, depending on recording stability. Paw lift-off from the ground was monitored using a high frequency carrier signal applied to a copper contact plate, and paw entry into the tube was detected by an infrared emitter–detector pair placed at the mouth of the tube. A potentiometer monitored the position of the tube. All data were stored on digital audio tape (DAT) for off-line analysis.

Testing of the responsiveness of the cerebellar neurones to a full-field visual stimulus was performed on as many cells as possible at the end of the recording session while the animal was sitting quietly, since this was a useful indication of responsiveness to an intense but behaviourally unimportant visual stimulus. A full-field flash stimulus (duration 8 μs) from a photic stimulator placed 50 cm away was delivered at irregular intervals. Experiments were performed in a well-lit room; thus the flash was a less strong stimulus than had the room been dark or dimly lit. A sheet of white paper over the flash screen acted as a diffuser, which also cut down the intensity. The animal had previously been habituated to such stimuli and, as a result, no startle movements or eye blink responses were seen. Whenever possible, attempts were also made to define any peripheral receptive field for somatic afferent inputs generated by manually delivered stimuli such as brushing of hairs, tapping of skin, palpation of muscle and the passive movement of joints.

Data analysis

All signals were digitized off-line by customized software running on a CED 1401 Plus computer interface unit (Cambridge Electronic Design, Cambridge, UK) at

200 Hz, together with unitary potentials discriminated and converted into 5 V pulses by a time–amplitude window discriminator. Using the Spike2 analysis package, peristimulus time histograms (PSTHs) with bin widths of 5 ms were constructed around: (1) delivery of flash stimulus; (2) start of target movement; (3) 'GO' signal; (4) end of target movement (i.e. target stop); (5) the reaching limb movement; and (6) right- and leftward saccadic eye movements. All histograms which were generated from $>$ 20 trials were analysed. The criterion for significant modulation was set at a discharge frequency (in at least 2 successive bins) more than two standard deviations above or below the mean level of discharge in the preceding 200 ms 'control' period (cf. Marple-Horvat *et al.* 1998). Onset latency was calculated to the leading edge of the first significant bin. Latency to onset and peak, duration of modulation, and peak frequency change from mean level in the control period were calculated. Individual histograms of a group of cells were sometimes combined to produce a single profile reflecting the activity of all the neurones in the group (e.g. for the combined activity of all Purkinje cells around the start of tube movement). Such pooled activity profiles could be compared in the same way as individual histograms.

Histological verification of recording sites and labelling studies

In one cat upon termination of the experiment (typically 8 weeks after the initial operation) the animal was re-anaesthetized as described above, and bidirectional axonal transport tracers were injected at certain mediolateral and rostrocaudal locations of the chamber in which most of the microelectrode tracks were made, to permit identification of the afferent and efferent connections of the cortical regions from which neuronal recordings were obtained. The anterograde and retrograde tracers used in each injection were fluorescently tagged dextran amines and fluorescently tagged beads, respectively. One injection mixture contained a 20% solution of Fluoro-Ruby (Molecular Probes, Eugene, OR, USA) combined with red beads (Lumafluor, New City, NY, USA), while the second injection mixture contained a 20% solution of Fluoro-Emerald (Molecular Probes) combined with green beads (Lumafluor). The tracer combination was delivered hydraulically via a glass micropipette (tip diameter 20 μm) attached to a 1 μl syringe (Hamilton, Bonaduz, Switzerland). In each case 200–400 nl of the tracer mixture was delivered 0.5–1 mm below the surface of the floor of the chamber. Following a survival period of 7 days to allow transport of tracers to occur, the animal was deeply anaesthetized with sodium pentobarbitone (Sagatal, 60 mg kg^{-1}, I.P.; Rhone Merieux, Harlow, UK) and perfused transcardially with 1 l of heparinized 0.9% saline rinse followed by 2 l of 4% paraformaldehyde fixative and then 2 l of 0.2 M phosphate buffer (pH 7.4) containing 10% sucrose. Subsequently, the brainstem and cerebellum were removed together and immersed in 30% sucrose at 4°C and allowed to sink overnight. Following this, the cerebellum and brainstem were separated, and a freezing microtome was used to cut the brainstem and cerebellar blocks into 50 μm transverse and sagittal sections, respectively. Two series of alternate sections were collected; one series was for analysis of fluorescent material, while the second series was Nissl stained with Cresyl Violet and used to permit reconstruction of microelectrode tracks.

Following a period of productive single unit recording, the other two cats were deeply anaesthetized with barbiturate, perfused, and the cerebellum cut, stained and viewed as described above.

For mapping of fluorescent labelling, sections were inspected with a Leica DMRB microscope fitted with a 50 W mercury UV light source. Red fluorescent labelling was viewed with an N2.1 filter block (Dichroic mirror 580 nm, band pass (BP) 515–560 nm, long pass (LP) 580 nm), while green fluorescent labelling was viewed with an H3 filter block (Dichroic mirror 510 nm, BP 420–490 nm, LP 520 nm). In the medulla and pons where axonal transport had occurred, the numbers of retrogradely labelled inferior olivary neurones were counted and their positions charted onto standard diagrams. In the cerebellum the locations of any Purkinje cell axonal terminations labelled with orthogradely transported Fluoro-Ruby or Fluoro-Emerald were charted on a set of standard parasagittal sections of the deep cerebellar nuclei, as described in previous studies (Trott & Armstrong, 1987; Edge *et al.* 2003).

Results

Extracellular recordings were obtained from a total of 86 cells (32, 35 and 19 from each cat) and 67 (78%) were visually responsive. Of the 86 cells, 63 (73%) were identified as Purkinje cells, nine were mossy fibres, four were putative Golgi cells and ten could not be classified.

Discharge patterns of Purkinje cell neurones in response to visual events

The simple spike activity of 63 Purkinje cells was studied against as many of the four visual events (flash, target onset, 'GO' and target offset) as possible. A Purkinje cell with less than 20 trials for a particular event was excluded from the analysis. As a result, some visual events do not include the total number of Purkinje cells (i.e. 63) studied. In addition to the individual response histograms, histograms of a group of cells were combined to produce profiles of the overall modulation in this restricted cortical region.

Testing of simple spike responsiveness to a full-field flash occurred at the completion of the reach–retrieval task, while the animal was sitting quietly. Such a stimulus was a useful indicator of responsiveness to an intense but behaviourally unimportant visual stimulus. Thirty-eight Purkinje cells were adequately tested against the flash stimulus, of which 25 (66%) modified their discharge. Nineteen of these Purkinje cells responded to the flash stimulus with a phasic increase in simple spike discharge rate (Fig. 2*A*), while the remaining six cells responded to flash with a reduction in simple spike discharge rate (Fig. 2*B*). No startle or eye blink responses were seen during presentation of the flash stimulus, indicating that the changes in neuronal activity were most probably associated with photic stimulation.

Fifty Purkinje cells were tested for simple spike visual responses to the start of tube movement irrespective of direction. Of the 50 tested, 23 (46%) displayed a change in simple spike discharge rate. Eight cells displayed a phasic decrease in activity (Fig. 2*C*). The remaining cells (15) exhibited an increase in discharge rate which could be phasic (Fig. 2*D*), or an initial phasic response followed by a sustained tonic increase (phasic–tonic, Fig. 2*E*); the tonic component is particularly clear in the pooled discharge profile (Fig. 2*F*).

To determine whether the 23 simple spike responses to the onset of target motion were directionally sensitive, PSTHs were aligned to the onset of target motion rightwards across the cat's visual field (the direction in which a 'GO' signal might be given) or to the start of target motion in the return leftwards direction at the start of the return travel to the target's initial location. Ten cells (10/23, 44%) were responsive to movement onset in both directions, of which eight cells responded to both movement directions, doing so in the same way. The two remaining bidirectional cells exhibited reciprocal responses to the two movement directions (increased discharge rate when the target was moving in one direction and reduced discharge rate when it was moving in the opposite direction). Thus, almost half the cells were not directionally exclusive in their responses. The remaining 13 cells (13/23, 56%) were unidirectional. Ten cells were responsive to target onset only in the rightwards direction, while three cells that were responsive to start of target movement in the return direction gave no response to movement onset in the rightwards direction.

Forty-six Purkinje cells were tested against the end of target movement (target stop), either because it had reached the end of its travel in 'no-GO' trials, or at the moment the cat's paw entered the tube at the end of a successful reach. Ten (22%) of these cells were responsive. Half of the responses were an increase in discharge rate (Fig. 2*G*), while the other half were inhibitory.

Table 1 summarizes the response parameters for the simple spike responses to visual events. Responses to the flash stimulus and the start of target movement had several common features. The majority of responses were excitatory. Onset latencies were short; the mean latencies at which a significant change (calculated as $\pm$ 2 S.D. of mean discharge rate during control period) in simple spike discharge occurred was between 33 and 46 ms. Peak modulation occurred between 48 and 58 ms, and was often a doubling or trebling of discharge rate. In contrast, simple spike responses to the target stopping displayed relatively late mean onset and peak modulation latencies compared to flash and target onset events, and only half the responses to the target stopping were excitatory.

A total of 33 Purkinje cells were adequately tested against the 'GO' signal (the signal to reach to the tube for the food reward). In order to exclude the possibility that the animal anticipated the 'GO' signal, PSTHs for the responsive Purkinje cells were confined to trials in which a successful reach was made, but excluded trials where the animal lifted its paw before the 'GO' signal or began lifting its paw less than 200 ms after the 'GO' signal. Fourteen cells (14/33, 39%) responded with modulation, the great majority (12) with an increase in discharge rate (Fig. 2*H*). Purkinje cells responsive to the 'GO' signal had longer onset latencies, longer durations and later peak modulations in comparison to the responses to the flash and target onset (Table 1). However, the excitatory responses to the 'GO' signal were approximately two times larger than the modulation of the excitatory responses to other visual events.

Complex spike activity could only be studied in a smaller sample of 18 cells, owing to the difficulty of reliably separating it from the more numerous simple spikes. Of these cells for which a characteristic complex spike composed of an initial spike followed by smaller secondary wavelets (see inset in Fig. 3*A*) could be isolated and analysed, 14 were tested against the flash stimulus, and eight (57%) were responsive. All complex spike responses of Purkinje cells to flash stimulus displayed a clear, strong increase in activity (Fig. 3*A*). Of the 11 Purkinje cells whose complex spike activity was tested for the start of target movement, two (18%) were responsive and exhibited an increase in discharge (Fig. 3*B*). Only target onset in the rightwards direction modulated complex spike activity. Ten Purkinje cells were tested against the 'GO' signal, with three displaying an increase in complex spike occurrence (Fig. 3*C*). No response was seen to the end of target movement (7 cells tested). The response parameters are given in Table 1. Onset latencies for the flash and target onset were short, with longer latencies for complex spike responses to the 'GO' signal. The flash and 'GO' signal were, however, more potent on average than the response to target onset; peak frequency change was twice as large for the flash stimulus, whereas the 'GO' stimulus was often a trebling of discharge rate.

Of the six flash-responsive Purkinje cells for which both complex spikes and simple spikes could be isolated, three

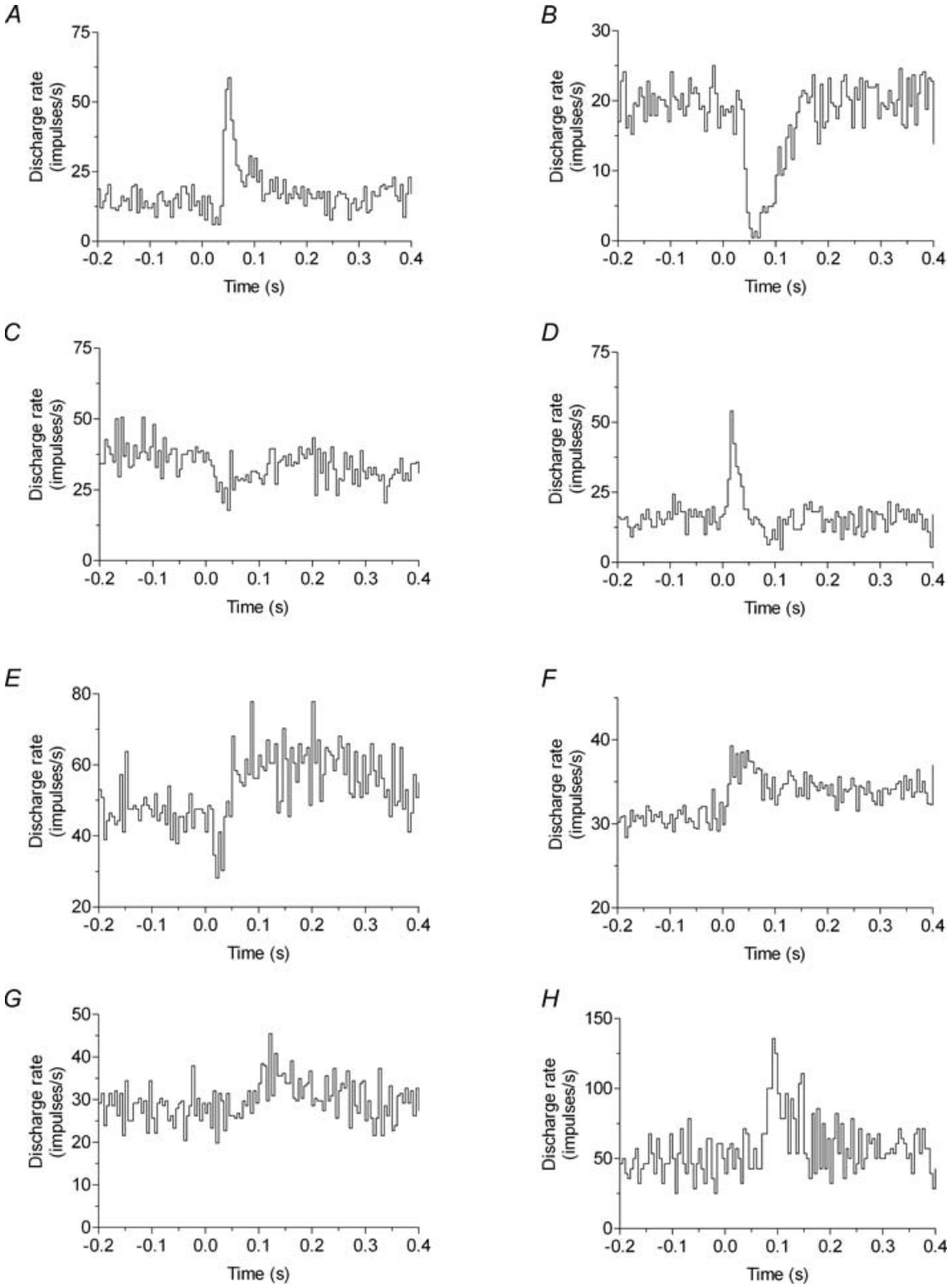


Figure 2. Purkinje cell simple spike activity in response to visual events
Each PSTH with a significant increase or decrease in firing rate is averaged around each visual event, which occurred in each case at time zero. Representative examples are shown of phasic increase (*A*) and decrease (*B*) in simple spike activity in response to the flash stimulus. Examples are shown of phasic decrease (*C*) and increase (*D*) in simple spike activity in relation to time of onset of tube movement. *E*, representative example of tonic increase in simple spike activity averaged around the start of tube movement. *F*, pooled activity profile, representing the overall modulation of all Purkinje cells whose simple spike activity displayed an increased significant tonic response

Table 1. Measures of neuronal activity during presentation of the flash stimulus and during visually guided reaching

	n	Onset latency (ms)	Duration (ms)	Peak latency (ms)	Peak frequency change (impulses s^{-1})
Purkinje cell simple spikes					
Flash excitatory	19	40 ± 30	35 ± 24	48 ± 24	31 ± 19
Flash inhibitory	6	33 ± 20	98 ± 36	54 ± 17	−22 ± 9
Target onset excitatory	15	46 ± 39	41 ± 21	58 ± 51	22 ± 10
Target onset inhibitory	8	46 ± 28	46 ± 41	55 ± 32	−15 ± 4
'GO' signal excitatory	12	66 ± 27	61 ± 51	105 ± 43	50 ± 17
'GO' signal inhibitory	2	63 ± 32	60 ± no S.D.	163 ± 103	−27 ± 16
Target stop excitatory	5	95 ± 51	123 ± 98	166 ± 95	24 ± 11
Target stop inhibitory	5	71 ± 52	68 ± 60	251 ± 169	−24 ± 7
Right EOG excitatory	8	−1 ± 74	72 ± 39	24 ± 67	28 ± 14
Right EOG inhibitory	1	10	20	25	−16
Left EOG excitatory	2	30 ± 56	60 ± no S.D.	118 ± 180	31 ± 12
Left EOG inhibitory	1	15	25	20	−10
Purkinje cell complex spikes					
Flash excitatory	8	59 ± 25	36 ± 17	69 ± 21	11 ± 6
Target onset excitatory	2	48 ± 17	58 ± 11	58 ± 11	6 ± 3
'GO' signal excitatory	3	85 ± 13	53 ± 13	100 ± 27	16 ± 7
Non-Purkinje cell					
Flash excitatory	7	36 ± 20	46 ± 37	51 ± 24	25 ± 19
Target onset excitatory	9	23 ± 13	38 ± 20	35 ± 15	36 ± 43
'GO' signal excitatory	6	64 ± 57	41 ± 35	118 ± 82	71 ± 58

Values are means ± S.D.

demonstrated convergent visual inputs by both mossy fibre and climbing fibre afferent pathways in that they yielded both simple spike and complex spike responses to the flash stimulus (Fig. 4). In such cases, both responses were an increase, and had similar latencies to onset and peak modulation.

Discharge patterns of non-Purkinje cell neurones in response to visual events

Recordings were obtained from a further 23 cerebellar cortical neurones: four putative Golgi cells, nine mossy fibres and ten neurones which could not be categorized. Of the non-Purkinje cell neurones responsive to the flash stimulus, two were Golgi cells, four were mossy fibres and one was not classified. All responded with increased discharge rate to the flash stimulus. Four mossy fibres, three Golgi cells and two unclassified cells exhibited an increased discharge rate in response to target movement. Of the non-Purkinje cell neurones tested against the 'GO' signal, two mossy fibres and four unclassified cells exhibited an increase in their discharge rate. Since these 23 cells comprise a small proportion of the entire cell population and are not sufficient in number to form viable groups when subclassified according to their responses and types, these cells were pooled together. The responses of these cells were similar to the simple spike responses of Purkinje cells (see Table 1).

Activity of Purkinje cells in relation to eye movements

Electro-oculogram recordings of eye movements were obtained whilst recording the activity of 28 Purkinje cells. Of these, ten (36%) were responsive to the onset of saccadic movements. Seven Purkinje cells modulated their activity in relation to rightwards saccades, six with an increase and one with a decrease (Fig. 5*A* and *B*); one Purkinje cell modulated its activity in relation to leftwards saccades, with an increase in discharge rate (Fig. 5*C*). Two Purkinje cells among the ten modulated their activity in relation to both leftwards and rightwards saccades. Of these two cells, one displayed an increase in activity in relation to

To the target movement (Student's paired *t* test, $P < 0.05$, $n = 15$). *G*, example of a simple spike response to the termination of target movement. *H*, representative example of simple spike response to the 'GO' signal. Bin widths for each PSTH are 5 ms and are composed of the following number of trials: 235, 447, 304, 222 and 185 for *A*–*E*, respectively, and 40 for *G* and 342 for *H*.

saccades in both directions, whereas the remaining cell modulated reciprocally, increasing its activity in relation to rightward saccades and decreasing its activity in relation to leftward saccades. The response parameters in relation to saccadic eye movements are presented in Table 1. The majority of cells displaying a modulation in simple spike activity in relation to saccades had response latencies that preceded saccade onset by 40–5 ms in seven cases (Fig. 5*A*) and followed it by 5–160 ms in three cases. Purkinje cells for which complex spikes could be isolated and non-Purkinje cells did not exhibit responses to eye movements.

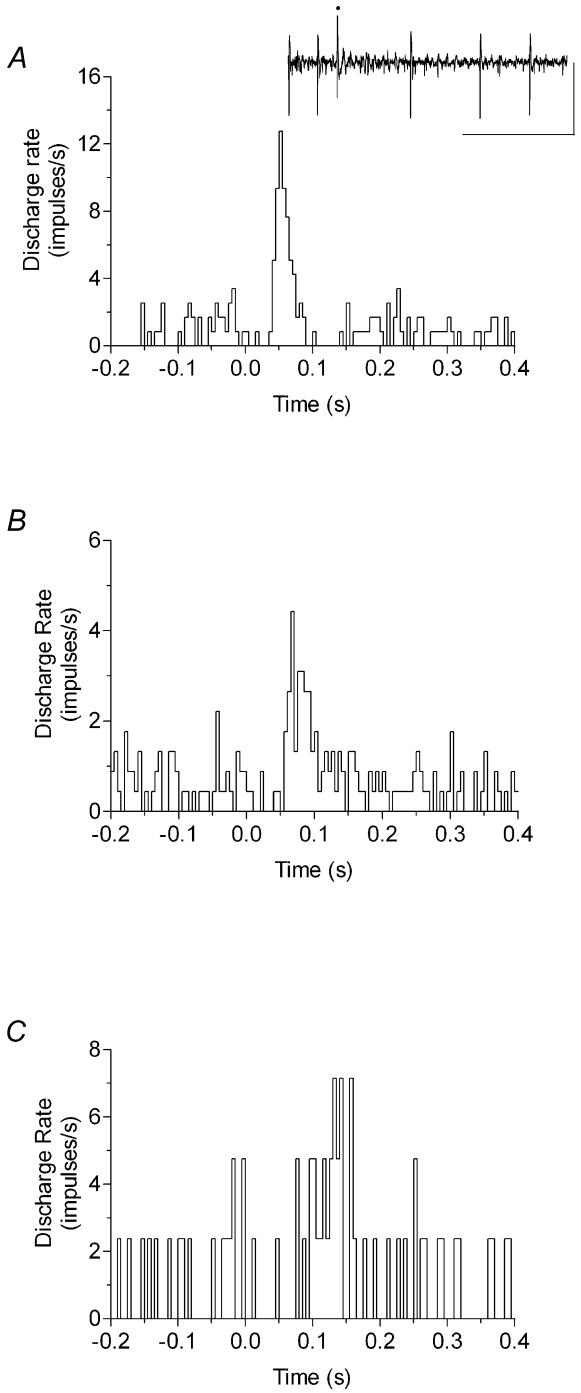


Figure 3. Representative examples of significant complex spike activity averaged around the flash stimulus (*A*), onset of target movement (*B*) and the 'GO' signal (*C*)
Each visual event occurred at time zero. Bin width is 5 ms and number of trials is 235, 279 and 85 in *A*, *B* and *C*, respectively. Inset in *A* displays the complex spike waveform, marked by a dot; voltage scale bar is 0.5 mV and time scale bar is 50 ms.

Activity of Purkinje cells in relation to limb movements

Every effort was made to localize for the cell under investigation a peripheral receptive field for somatic afferent inputs generated by manually delivering mild mechanical stimuli, such as brushing of hairs, skin taps, palpation of muscles and passive movement of joints. Previously, such testing easily identified the presence of a limb receptive field in other cerebellar cortical regions (Marple-Horvat *et al.* 1998). No receptive fields related to forelimb manipulation and stimulation could be found.

In the experimental protocol, the 'GO' signal was followed by the cat responding by lifting his paw off the ground. Since there is a possibility that some lateral cerebellar cells may also modulate their activity in relation to this movement and contribute to the duration of the responses, the timing of the onset of muscle activity was studied in relation to the 'GO' signal in cells for which EMG records were available. Of six such cells, four modulated their discharge rate in relation to the 'GO' signal with onset latencies of 25–80 ms, and duration of modulation ranging from 30 to 85 ms. The onset latencies of modulation in the EMG in relation to the 'GO' signal for these four cells were much longer, ranging from 75 to 430 ms. An example of one such cell is shown in Fig. 6*A*. First detectable activity in the EMG onset latency is 75 ms, but appreciable levels of activity only develop after 120 ms. Even at 75 ms the short latency strong modulation of

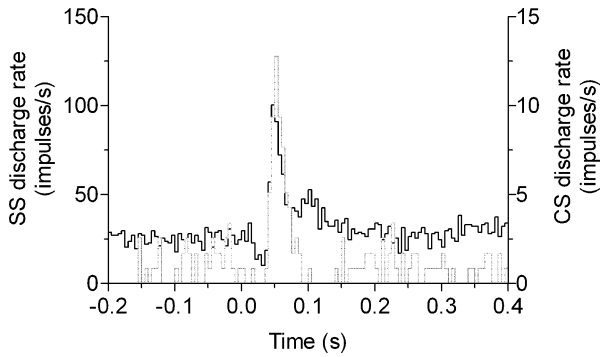


Figure 4. Example of a Purkinje cell for which both simple spikes (SS) and complex spikes (CS) could be isolated and yielded a response to the flash stimulus
Simple spike PSTH shown in black; complex spike PSTH shown in grey. Bin width is 5 ms and the PSTH is composed of 235 trials.

neural activity is over. Since there is no overlap either between the 'GO' signal and the modulation in EMG activity or between the neuronal modulation and EMG activity, it is likely that in most cases the response of the cells to the 'GO' signal, for which the average onset ranged from 30 to 75 ms, could not be attributed to muscle activity (although earlier modulation in EMG in other muscles cannot be excluded). The remaining two out of six cells had an onset latency of 55 ms in relation to the 'GO' signal; however, in these two cases elevated discharge rates beginning with the response onset were partly sustained for several hundred milliseconds (Fig. 6*B*). Although again the early, clearest modulation was over before appreciable EMG developed (∼200 ms), there is the possibility in these cells that although modulation started in relation to the 'GO' signal, modulation related to the movement may from 200 ms onwards have contributed to this late sustained high discharge rate. Such cells, neither solely visual nor solely motor in their properties, might best be regarded as visuomotor. Their small number nevertheless emphasizes the predominance of sensory (visual) over movement-related responses.

Target motion and velocity

As well as the initial monophasic excitation described above for simple spike responses to the start of target movement, a sustained, tonic increase in discharge rate was observed when the target was moving compared to when it was stationary (as is evident when the responses were pooled; see Fig. 2*F*). Since the target moved at constant velocity, this transition from 'low' to 'high' discharge level correlates with the velocity–time profile of target motion (a step increase from zero to a constant level). Thus, the simple spike activity of these Purkinje cells could encode target velocity, especially since the reverse transition was seen when the target stopped moving, with discharge rate reverting to a lower level. The relationship between tonic discharge rate and target velocity was analysed by comparing the mean discharge rate during a 200 ms period immediately preceding the start of target movement with the mean discharge rate for the period 100–300 ms into its movement (which excluded the initial phasic response and preceded delivery of the 'GO' signal) while the target was moving at different constant velocities of 3.08, 6.16 and 12.32 cm s^{-1} rightwards across the visual field.

Among the 23 Purkinje cells whose discharge rate was altered during target movement, eight cells accumulated sufficient trials against more than one target velocity in order to permit investigation into the relationship between their tonic discharge rate and target velocity. The mean (± S.E.M.) discharge rate for a control period which corresponded to a 200 ms period preceding the start of the target movement and for the period 100–300 ms into the target's movement was calculated from PSTHs generated for each target velocity. The minimum number of trials for each target velocity was 20; however, the majority of PSTHs were generated from 30–40 trials. Seven cells had a 'preferred' target velocity rather than a linear relationship with velocity ($P < 0.05$, one-way ANOVA with Tukey's

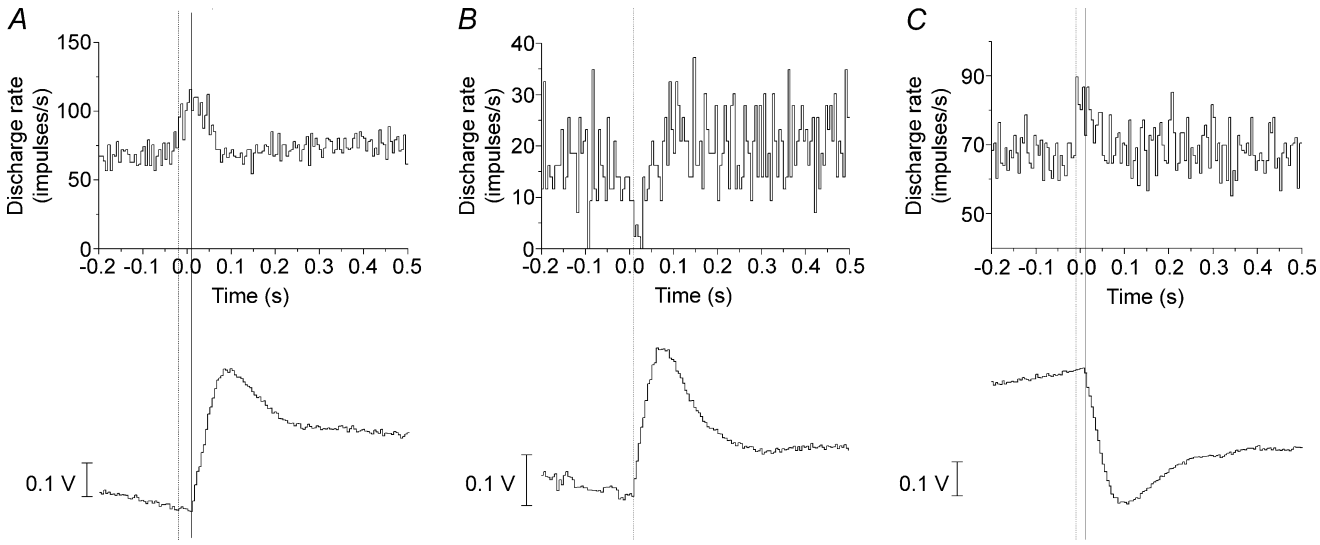


Figure 5. Simple spike activity in relation to eye movements
Representative examples of significant PSTHs averaged in relation to time of onset of horizontal rightward (*A* and *B*) and leftward eye movement (*C*). Each histogram is accompanied (below) by the corresponding saccade profile. Simple spike and saccade onset latencies are shown by dotted and continuous line, respectively, in *A* and *C*. Simple spike and saccade onsets in *B* coincide and are shown by the dotted line. The number of trials for *A*–*C* are 310, 132 and 272, respectively. Bin width is 5 ms.

post hoc analysis; Fig. 7*A*–*C*), while the remaining cell was significantly modulated for two target velocities (Fig. 7*D*). All eight cells responded to unidirectional target movement in the rightwards direction, i.e. the direction in which a 'GO' signal might be given.

To analyse the possibility that the difference in discharge rate seen during target motion was related to concurrent eye movements made to track the target, the activity of 32 Purkinje cells was studied in relation to both target motion and eye movements, i.e. cells were tested for both saccade relatedness and target motion using the criterion for significant modulation as described earlier (2 successive bins ± 2 S.D. from mean discharge rate during the control period). Only four cells (13%) were related to both, seven (22%) were related only to eye movements and ten (31%) only to target motion. Eleven cells (34%) were neither saccade nor target motion related. Thus, there were only four cells in which activity related to eye movements could have been wrongly interpreted as responsiveness to the visual stimulus.

Number of visual events to which lateral cerebellar neurones responded

To examine the overall picture of the extent of visual responsiveness of lateral cerebellar cortical neurones, the number of visual events (flash, start of target motion, 'GO' signal and termination of target motion) that a cell was tested for and responded to was determined for each cell. The proportion of neurones that responded to all events against which they were tested fell with increasing number

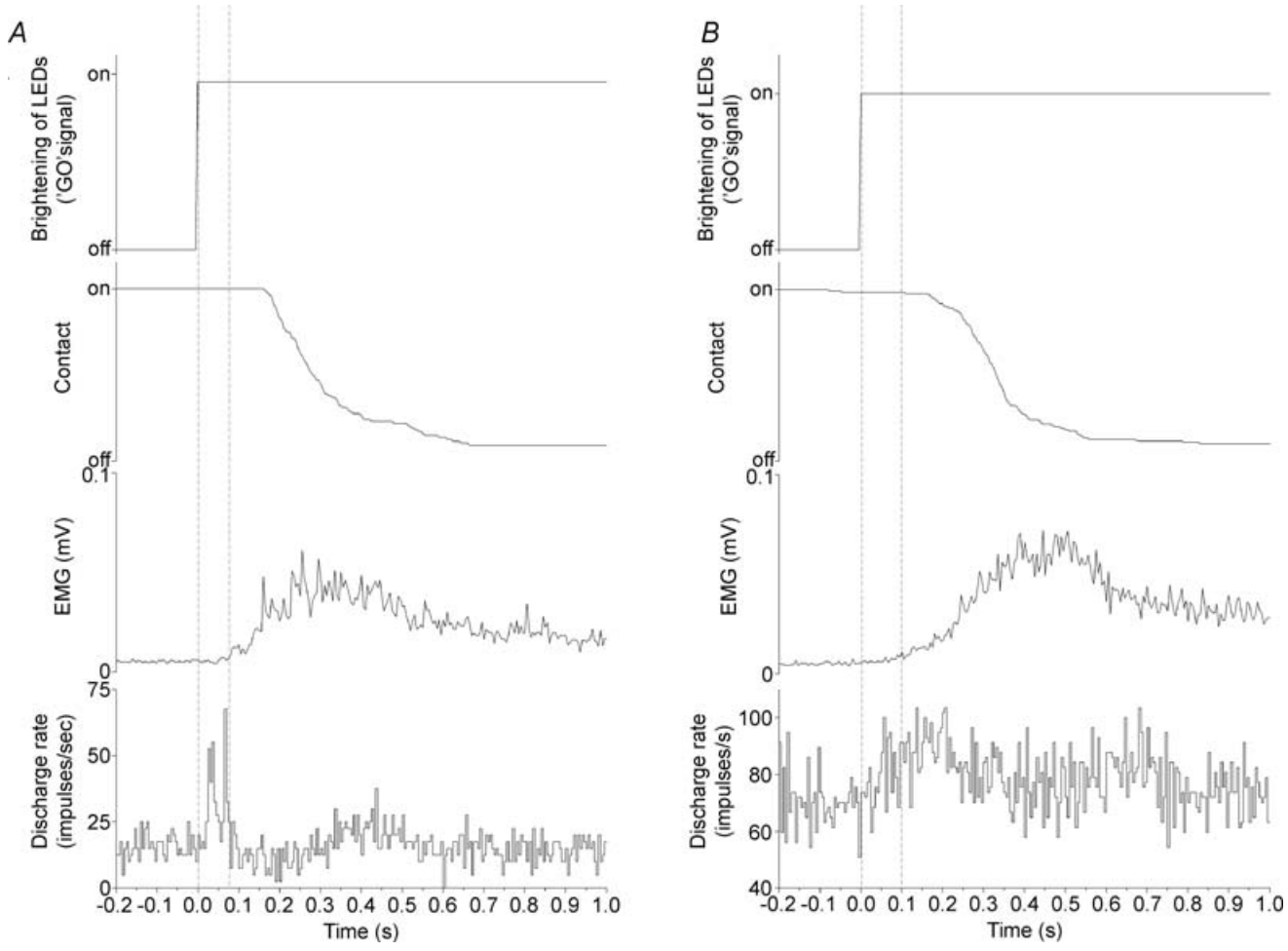


Figure 6. Relationship between the 'GO' signal, footlift, EMG activity and Purkinje cell activity (top to bottom traces, respectively)
A, an example of a Purkinje cell aligned to the 'GO' signal (first dashed line) which had an EMG onset latency of 75 ms (second dashed line). In this example, there is no overlap between the 'GO' signal and the modulation in EMG activity. Purkinje cell response latency is 25 ms. *B*, example of a Purkinje cell which had a longer lasting response to the 'GO' signal (first dashed line) with an onset latency of 55 ms. Electromyogram onset latency (second dashed line) was approximately 100 ms.

of events (Fig. 8*A*). Fifty-eight cells were tested against three or more events, and 17 (29%) responded to all events tested; 36 cells were tested against all four events, and seven (19%) responded to all. The great majority of cells were therefore selective in their responsiveness. An example of a Purkinje cell responding to more than one visual event is shown in Fig. 8*B*.

Localization of visually responsive neurones

The mediolateral and rostrocaudal stereotaxic locations of recordings obtained in each animal are shown in Fig. 9*A* and *B*. Recordings were made from a precisely corresponding region: a 2.25×2 mm area located 10.25–12.5 mm from the mid-line and 4–6 mm behind the rostral border of the crus I folia. Maps of the locations of the subpopulations of cells that responded to flash stimulus, onset of target movement, and the 'GO' signal are illustrated in Fig. 9*C*, *D* and *E*, respectively. Cells responsive to these different visual events did not cluster within this limited area of cortex, but rather were scattered throughout the area from which recordings were obtained.

In one animal two bidirectional axonal transport tracers were injected into the area coinciding with the parts of the cortex in which most of the microelectrode tracks were made during the recording sessions, and in one outlying microelectrode track to permit identification of the afferent and efferent connections of this cortical region. Fluoro-Emerald combined with green beads was placed into the area where most of the recordings where obtained, whereas the combination of Fluoro-Ruby and red beads was placed at the location of the one outlying (caudomedial) track (Fig. 9*B*). Figure 10*A* shows the distribution of retrogradely labelled cells in the contralateral inferior olive labelled with green beads. The majority of labelled cells (68%) were located in the ventral lamella of the principal olive (vlPO) between rostrocaudal (RC) levels 9.0 and 10.75. The remaining labelled cells were found in medial accessory olive (MAO) between RC levels 9.0 and 10.0. When examining the Fluoro-Emerald anterogradely labelled terminals (Fig. 10*B*), a greater preponderance of terminals were confined to the dorsal portion of the dentate nucleus and within the dorsal transitional region between nucleus interpositus anterior (NIA) and dentate nucleus. Further medially, sparse terminal labelling was also located between and in NIA and nucleus interpositus posterior (NIP). Figure 10*C* shows the olivary cells retrogradely labelled with red beads. Ninety-two per cent of cells labelled were confined to the medial dorsal accessory olive (DAO) between RC levels 9.0 and 10.25. The few additional labelled cells were located in the rostral MAO between RC levels 9.25 and 9.5. The location of Fluoro-Ruby anterogradely labelled terminal fields is illustrated in Fig. 10*D*. Terminal labelling was found in the dorsocaudal part of NIA and within the dorsal

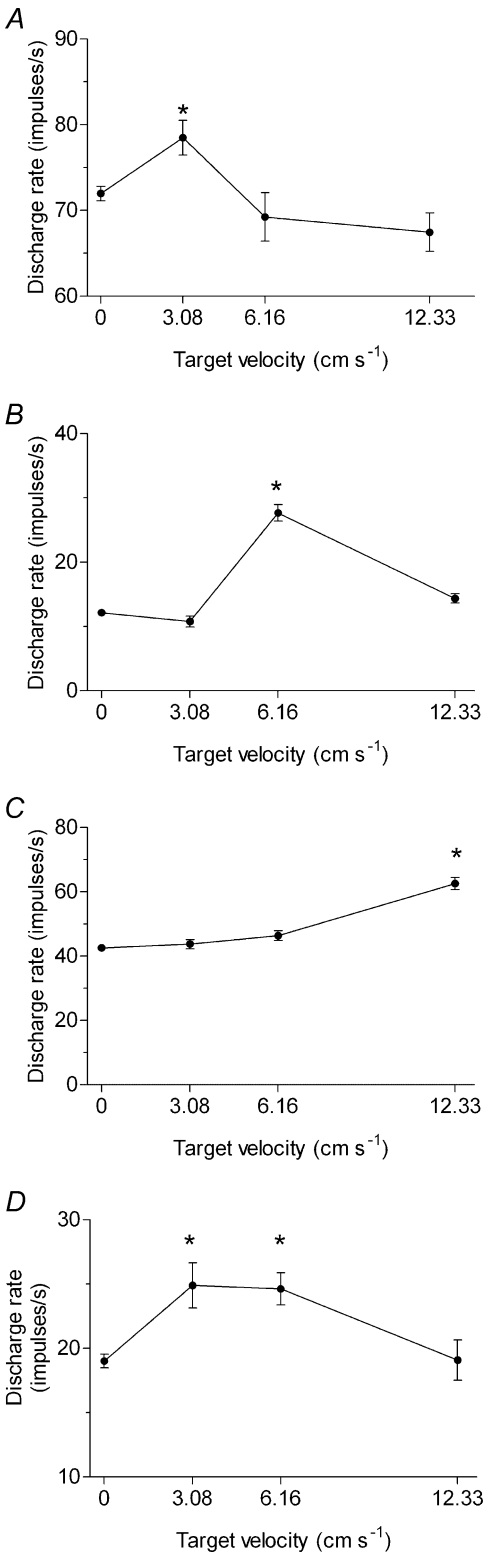


Figure 7. Relationship between Purkinje cell tonic discharge rates and target velocity
Examples of Purkinje cells that showed significant modulation ($P < 0.05$, one-way ANOVA) to a preferred target velocity of 3.08 (*A*), 6.16 (*B*) and 12.33 cm s^{-1} (*C*). One Purkinje cell was significantly modulated for two target velocities (*D*). Each data point represents mean tonic discharge rate ± S.E.M. *indicates discharge rate significantly higher than when target stationary ($P < 0.05$).

transitional region between NIA and dentate nucleus. Additional terminal labelling was also present in the dorso-rostral portion of NIP, although this was considerably sparser than in NIA.

The pattern of afferent and efferent labelling from injections placed in the cerebellar cortex indicates that the red injection site (i.e. the one outlying caudo-medial track) was primarily in the C3 cortical zone, with some spread to the neighbouring C2 zone, whereas the green injection site was mainly located in the D1 zone with some spread to the adjoining C2 zone. As the green injection site was placed within the main site of electrode penetrations, this defines the cortical region from which recordings had been made as the hemispheral D1 zone.

Discussion

The great majority of neurones (78%) were visually responsive. They lay within a precise region of crus I which received climbing fibres from the ventral lamella of the principal olive, and their axons projected to caudal dentate nucleus (Voogd & Bigaré, 1980; Rosina & Provini, 1982; Edge *et al.* 2003), identifying the region as the D1 zone.

Discharge patterns of lateral cerebellar neurones in relation to visual events

Most responsive neurones were Purkinje cells, and most of these responded to flash stimuli like cells more medially in crus I and lobulus simplex (Marple-Horvat *et al.* 1998), with increased discharge rate. Noteably, the region sampled (D1 zone) is rostrolateral to, and distinct from, other areas sampled (C2 and C3 zone) by Gruart *et al.* (1997) and Edge *et al.* (2003), where cells responsive to periorbital stimulation and related to blinks have been found.

A flash of light is a strong, brief stimulus, and these characteristics were reflected in the neuronal responses, which were sharp, brief excitations. The majority had onset latency > 25 ms (mean 40 ms). Appreciable numbers (9/25; 36%) had shorter latencies. Baker *et al.* (1976)

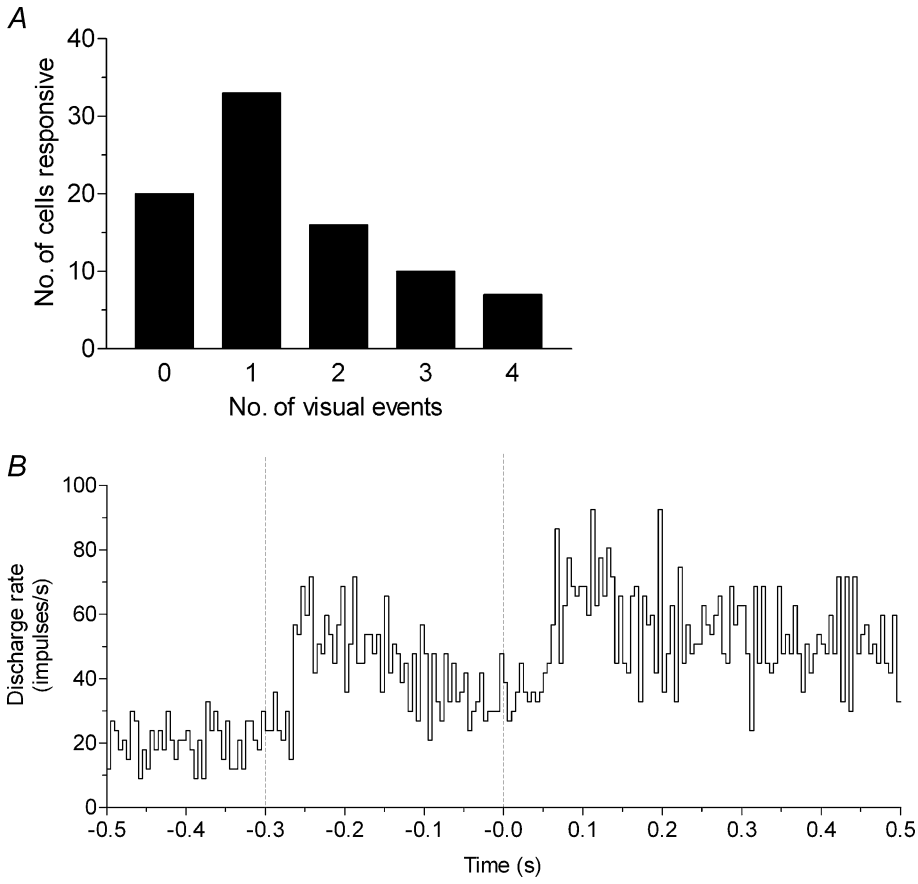


Figure 8. Extent of visual responsiveness of lateral cerebellar cortical neurones
A, number of cells responding to certain numbers of visual events. *B*, example of a Purkinje cell that responded both to the start of target movement (first dashed line at −0.3 s) and the 'GO' signal (second dashed line at time zero). Number of trials is 65. Bin width is 5 ms.

similarly demonstrated pontine visual cells responding < 25 ms after a flash stimulus. The pons is a major link between visual centres and the cerebellum (Robinson *et al.* 1984; Glickstein *et al.* 1985, 1994; Stein & Glickstein, 1992; Glickstein, 2000). Cortical area 18 and the lateral suprasylvian area, which project to the pons (Bjaalie, 1985, 1986; Kato *et al.* 1988) also respond to flash stimuli with short latency (Doty, 1958; Berkley *et al.* 1967). A very fast path conveying visual information to the cortical D1 zone could therefore have its origins in these areas, though we offer here no experimental evidence for this.

Twenty-three Purkinje cells responded to onset of target movement, the first task event; 13 were directionally exclusive. We did not study a range of directions; the target always moved rightwards whilst the cat was waiting for the 'GO' signal. At the end of each trial, the target moved leftwards towards its starting position. The ten cells responsive to target onset in both directions show that a considerable proportion of cells were not directionally exclusive. The more modest modulation and lower number of cells responding to the return movement is probably because this occurred when the animal had less need to attend to the target, because it was eating its food reward after a successful reach, or it was a 'no-GO' trial and the cat was not therefore expecting a reward. The two cells that responded to start of target movement in the return direction only would seem to be highly directionally selective, since they responded only to movement in the behaviourally unimportant direction; a larger sample might discover more such responses. Directional selectivity of pontine visual cells, input neurones to hemispheral cerebellar cortex, was routinely seen by Baker *et al.* (1976), so our findings are not unexpected.

In all neurones, a broader, longer latency response was seen following the 'GO' signal than other visual events. Longer latencies could reflect the fact that brightening of the already glowing LEDs is a less intense stimulus, but responses were overall larger for the 'GO' signal, indicating that it was nonetheless a more effective or potent stimulus.

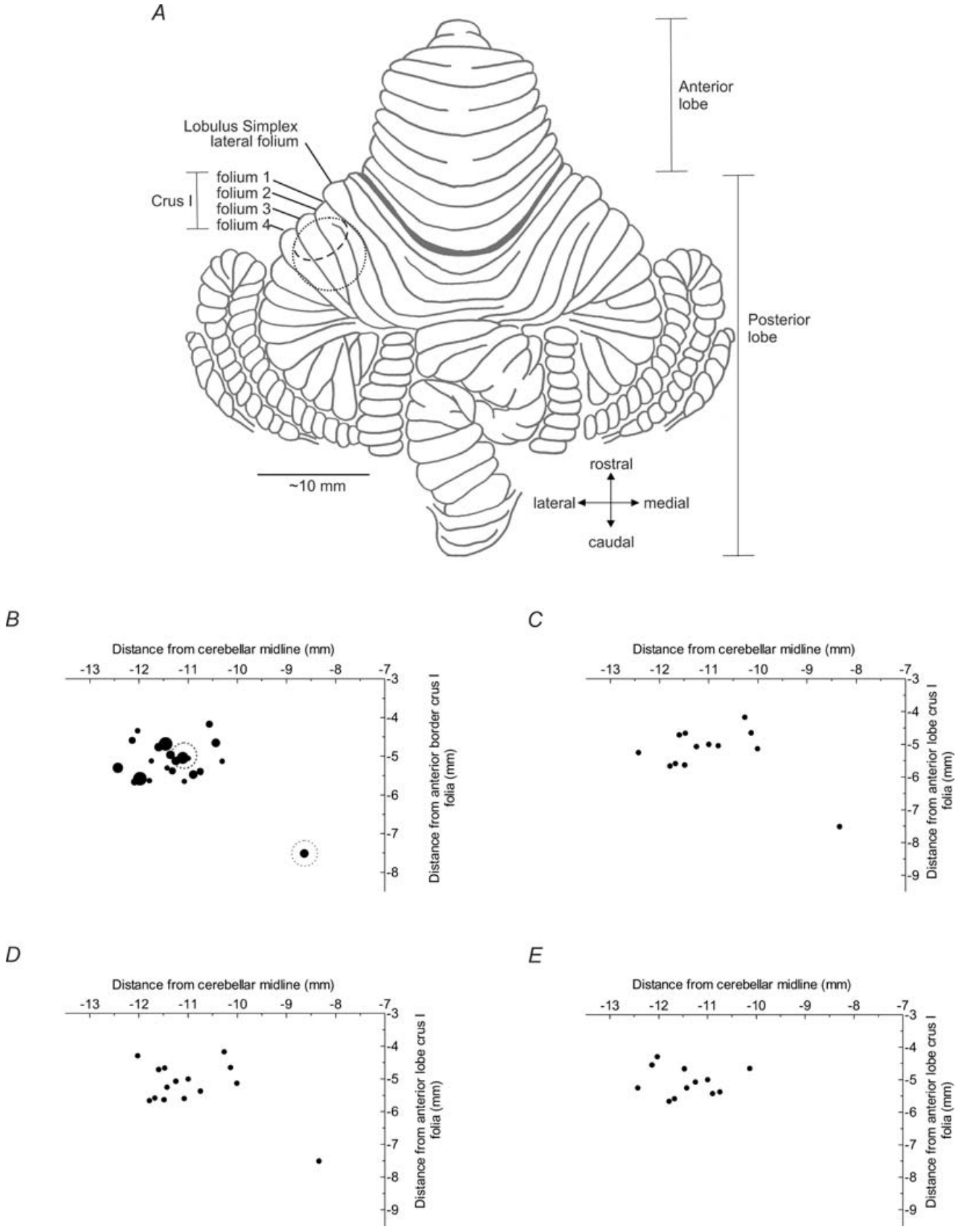
Average onset latency to this visual cue (66 ms) is compatible (bearing in mind the range of values in both studies) with the study by Chapman *et al.* (1986), in which the onset latency of dentate neurones to a visual cue for an elbow movement in monkeys was $\sim$90 ms. Purkinje cells in the D1 zone provide input to the caudal part of the dentate nucleus, where Chapman and co-workers recorded most of their visually responsive neurones.

Our finding of many responses to the 'GO' signal contrasts with a study by Strick (1983), which involved lights as cues to instruct monkeys whether they should pull or push a lever in response to displacements imposed via a torque motor. Among 46 dentate neurones responsive to perturbation of the lever/arm, none responded to the visual cue. We suggest that this is because the cue was only abstractly related to the subsequent movement, rather than a direct target for it, since in a subsequent study a large number of dentate neurones gave short latency ($\sim$70 ms) visual responses to target lights (comparable to our own) when they were used by the monkey to guide a reaching forelimb movement (Mushiake & Strick, 1993).

Our target stopped moving either because the cat did not receive a 'GO' signal or owing to entry of the forepaw at the end of a reach. The scarcity of responses to the target stopping probably reflects the lack of attention on the target when the cat had not received a 'GO' signal. It would also seem that the end of a successful reach was a rather ineffective event. The longer response latencies and durations than for other visual events raise the possibility of these responses being related to another aspect of the task following shortly after the tube stopped. They might be related to vision of the limb, or to limb or paw movements as the cat grasped and retrieved the food reward. None of these cells, however, had a limb or paw somatic afferent receptive field. Therefore, modulation following termination of tube movement does not seem likely to result from inputs from limb mechanoreceptors affected by the act of grasping, leaving vision of the limb as the more likely explanation.

Response of Purkinje cells to target motion: what is being encoded?

While many Purkinje cells showed brief responses which precisely signalled visual events, half of those tested exhibited simple spike activity compatible with encoding additional information, specifically tonically altered discharge rate during on-going visual stimuli. A task-related visual event in a previous study (Marple-Horvat *et al.* 1998) was movement of a rung on which the cat subsequently stepped. The moving rung is similar to the moving tube in that it was a target for a visually guided forelimb movement. The simple spike responses of Purkinje cells in both tasks share similarities in onset and peak latency and in usually being excitatory with similar changes in discharge rate; also, in each case the response was maintained for as long as the target was moving. There were important differences between the two protocols; rung movement was short lived (160 ms) whereas even at the fastest speed the tube was moving for $\sim$500 ms (and at slower speeds proportionally longer). Also, the rung stopped well before the cat stepped upon it, whereas the tube continued to move until the cat's paw entered the tube mouth. But in each case, Purkinje cell activity modulated for as long as the target was moving. The tonic changes in discharge rate in this study might therefore reflect a need to encode an on-line description of the target's motion, because it will continue to move for some time, and will still be moving throughout the interceptive movement made by the animal.



Several studies suggest that Purkinje cell discharge can be tuned to a specific direction–speed combination, or 'preferred velocity' during visually guided tracking tasks (Mano & Yamamoto, 1980; Marple-Horvat & Stein, 1990; Coltz *et al.* 1999; Johnson & Ebner, 2000; see also Gomi *et al.* 1998 in relation to eye movements). Thus, cells that exhibited a sustained tonic response could be encoding the velocity of the visual target. Ebner and co-workers studied monkeys performing visually guided reaching (Fu *et al.* 1997) and found that Purkinje cell activity in lobules V–VI correlated with direction or distance of the reach, or target position. Zonal identity of the cells was unknown, but only cells related to target position were perhaps drawn from the D1 zone and perform a similar function to the neurones in our study. A more recent investigation (Coltz *et al.* 1999) involved tracking targets moving in different directions and at different speeds. Purkinje cells modulated their discharge in relation to specific combinations of speed and direction, suggesting that individual cells encode a preferred movement velocity (see also Johnson & Ebner, 2000). For most combinations studied, the population response of Purkinje cells accurately specified target velocity (Coltz *et al.* 2000). In our study, the altered tonic discharge rate during target movement is clearly compatible with encoding the speed at which the target was moving. Unfortunately, it was not possible to test all cells against all available velocities and, at most, only three velocities were tested. Although these factors limit interpretation, for the cells that we were able to test, each showed a different response to different velocities, and a 'preferred' velocity. These eight cells did not modulate activity during target movement in the reverse direction back to its start location, i.e. they were unidirectional.

Discharge of lateral cerebellar neurones in relation to limb movements

Greger *et al.* (2004) examined Purkinje cell activity in a 7×7 mm region of cortex traversed diagonally by the fissure separating lobulus simplex and crus I in monkeys during a visually guided reaching task. Four-fifths of the neurones modulated their activity in relation to reaching; 68% of those for reaching with both forelimbs. Between a quarter and a third of the cells modulated their activity differently for reaches to ipsilateral *versus* contralateral space, or for reaches at different speeds (and did so for reaches with either arm). Since activity was modulated irrespective of the limb used, and correlated with two motor parameters, they concluded that lateral cerebellar cortex exerts control over motor parameters that are *abstracted* from the effector limb. Although zonal topography was not established, it is likely (Fig. 1*B*, *op. cit.*) that this broad region spanned the C2, C3 and D1 zones. Given that the majority of cells in our study modulated their activity powerfully and with short latency to visual stimuli, possessed no limb receptive fields and did not modulate their activity (except for a few, weakly) in relation to reaching, and were restricted to a 2×2 mm region identified as the D1 zone in crus I, whereas Greger *et al.* (2004) report the inverse, limb movement-related activity but no short latency visual responses, it would appear that our study essentially sampled a different population with a different motor control function. It would be interesting to know whether the neurones described by Greger *et al.* (2004) possessed receptive fields in the limbs. In the cat C2 zone, cells have a simple spike receptive field in both forelimbs (Edgley & Lidierth, 1988). If the monkey is the same, and the subset of neurones that modulated to movement of both limbs was drawn from the C2 zone, that modulation might still relate directly to changes within the receptive field in the moving limb, rather than representing a level of abstraction.

Visually responsive neurones and internal models

Miall *et al.* (1993; see also Wolpert & Miall, 1996) have suggested that an internal model is constructed in the cerebellum that can predict the sensory consequences of a familiar movement. In a region of lateral cerebellar cortex where neurones neither possessed a receptive field in the arm nor discharged in relation to eye movements, directionally selective discharges were found that related to the motion of a visible cursor controlled by the monkey, i.e. the visual consequences of the movement, rather than the arm movement itself, which could be in the same or opposite direction (Miall, 1998; Liu *et al.* 2003).

Figure 9. Distribution of visually responsive cells
A, dorsal view of the unfolded cat cerebellum (modified from Larsell, 1953) indicating the approximate positions of the chamber (dotted line) and of the area where the recordings were made (dashed line). *B*, stereotaxic map of position of recording tracks from all 3 animals. Recordings were made from a precisely corresponding region: a 2.25×2 mm area located 10.25–12.5 mm from the mid-line and 4–6 mm behind the rostral border of the crus I folia. The size of the dot relates to how many responsive neurones were identified in a single electrode track, with the smallest size representing 1 cell and the largest size representing 6 cells. Dashed circles around the centre of the majority of microelectrode tracks and around the outlying track indicate where the combination of Fluoro-Emerald and green beads, and Fluoro-Ruby and red beads, respectively, were placed (See results for further details). Stereotaxic maps of locations of the subpopulations of cells that responded to the flash stimulus (*C*), onset of target movement (*D*) and the 'GO' signal (*E*) are shown.

We recorded from lobule VII (crus I), whereas Liu *et al.* (2003) recorded from lobules V–VI. But this does not mean there is no anatomical–functional overlap between the studies. Our recordings were from the D1 zone. Activity related to target movement and other visual events dominated, whilst limb receptive fields and activity related to eye movements were both rare. The neurones that Liu *et al.* (2003) describe as coding the visual consequences of movement also lacked limb receptive fields or activity related to eye movements, suggesting that those recordings

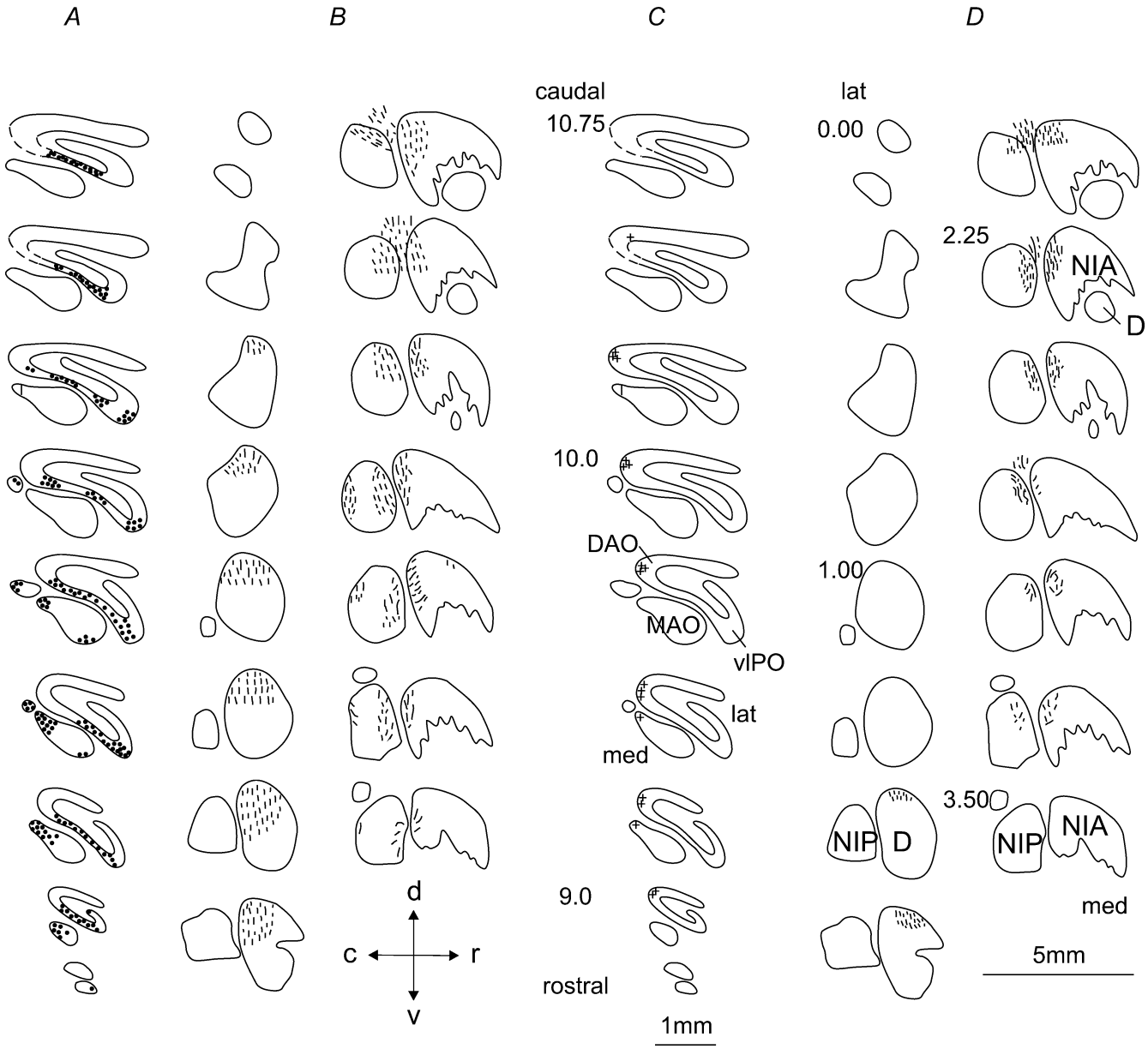


Figure 10. Input-output relations of cortical region revealed by bidirectional traces
Distribution of olive cell labelling and cerebellar nuclear terminal labelling after a tracer injection of Fluoro-Emerald combined with green beads (*A* and *B*) placed in crus I, coinciding with the parts of the cerebellar cortex in which most of the microelectrode tracks were made, and another injection of Fluoro-Ruby combined with red beads (*C* and *D*) placed in the location of the one outlying electrode track (See Fig. 9*B* for more details about injection site locations). The figure shows equally spaced transverse outlines of the inferior olive between anteroposterior levels 10.75 and 8.75, showing retrograde cell labelling. Numbers to the left of the equally spaced sagittal outlines of the cerebellar nuclei refer to the distance (in mm) of each level from the lateral edge of the dentate nucleus. Each dot and cross corresponds to one green and red retrogradely labelled cell, respectively. Anterograde-labelled axon terminal fields are shown by lines in the region of labelling. DAO, dorsal accessory olive; lat, lateral; m, medial; MAO, medial accessory olive; vlPO, ventrolateral principal olive; NIA, nucleus interpositus anterior; and NIP, nucleus interpositus posterior.

were also from the D1 zone (not C2 or C3) in lobules V–VI. Because of the continuity of the D1 zone through the different lobules, at least some of the neurones in the two studies may be functionally homologous.

The neuronal activity related to target movement in the present study might therefore be understood in two ways: *either* as passive coding of target motion by neurones driven by the visual stimulus *or*, since the target was familiar, an internal cerebellar model of its motion could have been constructed, so that these modulations represent the active operation of an internal model closely simulating target motion. One way of distinguishing between these possibilities would be to temporarily extinguish the target. Whilst invisible, the tonic increase in discharge rate would presumably disappear if those cells were being passively driven, whereas it might survive if centrally generated. The first results of such experiments do suggest that the activity of these cells betrays the operation of an internal model of target movement, in that the tonically increased discharge rate survives temporary visual denial of the target (Cerminara *et al.* 2004).

Visually responsive complex spike activity

Detection of visually evoked complex spike activity is an important finding, which indicates that climbing fibres supplying Purkinje cells well lateral in the hemisphere (10–13 mm) have stronger visual properties than those projecting more medially, 5–9 mm from the mid-line (cf. Marple-Horvat *et al.* 1998). Such responses confirm a recent demonstration that the D1 zone in crus I of the cat could be identified physiologically by optic tract stimulation (Edge *et al.* 2003). Complex spike activity in monkeys generated at the beginning of a visually guided reach encodes the location in space of the target (Kitazawa *et al.* 1998). Our observed complex spike activity could do the same.

Following Greger *et al.* (2004), Norris *et al.* (2004) examined complex spike discharges for the same population of Purkinje cells. A complex spike was only seen ahead of a saccadic eye movement, and was independent of any arm movements. Complex spike activity preceded by ∼50 ms saccades to a particular (preferred) location where there was a visual target, which had just appeared, either as its initial location, or by shifting there from elsewhere. The making of such an eye movement perhaps explains all of their findings. Although within trials this was always preceded by target appearance, occurrence of a complex spike was not tied in the same way to that event, since outside of trials complex spikes similarly preceded saccades made spontaneously into the preferred region of visual space, *but without any target being present*. The long latency of the complex spikes, ∼150 ms after target appearance within trials, also suggests that they do not represent visual responses. This contrasts with our few complex spike recordings, which were short latency (50 ms) visual responses, independent of any eye movements, and evoked by several visual events, including a full-field flash that occupied no particular location in the visual field and elicited no eye movement or other response. These purely visual complex spike responses were obtained from a small part of the identified D1 zone in crus I, and it is likely that our cells represent a different neuronal population.

Much of the above confirms two things: firstly that the cerebellar contribution to visuomotor control is probably crucial and multifacetted; and secondly that zonal identity is an important factor for the specific functional role of individual neurones and collections of neurones.

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